SCIENTIFIC PAPERS.

STUDIES ON THE LIGNIN AND CELLULOSE OF WOOD.*

BY PERLEY SPAULDING.

INTRODUCTION.

In a recent paper Potter † gives the results of some investigations upon the cellulose and lignin of the xylem of tree stems. By microchemical methods he found that cellulose occurs as a distinct lining layer in the walls of the wood fibers of perfectly healthy trees grown in various parts of England. Trees of *Quercus*, *Fagus*, *Aesculus*, *Salix*, *Ulmus*, *Alnus*, *Betula*, and *Fraxinus* were found by him to have cellulose occurring in this manner through all parts of the stem; that is, in both heart and sap wood at varying distances from the bark. He tested the action of boiling water upon the lignin of the cell walls of wood by cutting microscopic sections and placing them in water in a "boiling tube" and boiling for about two hours per day on consecutive days, the sections remaining in the water all of the intervening time. The lignin began to leave the walls in four to six hours of boiling in the sections of *Fraxinus* and similar results were obtained with other woods, twelve hours being the longest period of boiling which is definitely mentioned by him. Simple soaking of small fragments of wood in cold water was then tried and it was found that a substance was obtained which had

* A thesis presented to the Faculty of Washington University, in candidacy for the degree of Doctor of Philosophy, April, 1906. — Published by permission of the Secretary of Agriculture.

† Potter. Annals of Botany. **18**: 121–140. (1904).

been extracted from the wood and which gave lignin reactions with phloroglucin and thallin sulphate, thus indicating a partial delignification of the treated wood. He was led to conclude that, "It is found that a gelatinous thickening layer which reacts at once to the various color tests for cellulose occurs very commonly, though very irregularly, in the fiber walls of the xylem as a normal condition in a great number of perfectly healthy trees, in all localities and situations. It may have a very partial distribution, or may occur very generally and conspicuously through the stem, and may be present only in parts of the same annual ring. Sometimes this innermost layer is represented only by a thin lining, at others by a very broad band which appears swollen and occupies a large part of the lumen."

Also, "The presence of this unlignified layer in the wood fibers probably represents a stage of arrested development. Its general prevalence having been overlooked, the conclusion is inevitable that the occurence of cellulose which has been attributed to the action of fungi must to some extent be ascribed to conditions already present and the effect of any method of sterilization must also be taken into account. The delignification cannot be attributed to an enzyme secreted by fungi."

AIMS OF THE PRESENT INVESTIGATIONS.

This paper with its rather striking results and conclusions led to the investigations here detailed. Tests were made: first, to determine the prevalence of cellulose in the stems of the trees of America; second, to confirm or disprove the results of Potter's work in testing the effect of boiling water upon the lignin of wood; third, to test the relative solubility of the lignin of the spring and summer wood of the annual ring of growth; fourth, to come to some understanding of the conditions existing in wood

when used in testing the action of fungi upon its component parts.

MATERIALS USED.

The present experiments were performed with freshly cut wood taken from the stems of trees which, with but few exceptions, were above six inches in diameter. Two sets of experiments were carried on: the first, of a preliminary nature, included but a few species of wood; while in the second, woods of about forty of the more common and important timber trees were tested. Still later a number of other species were examined for the presence of cellulose without performing the boiling experiments.

The species of wood used in the preliminary experiments were:* *Populus tremuloidus* from New Mexico; *Pinus palustris* from Texas; *Sassafras Sassafras*, *Taxodium distichum* and *Nyssa aquatica* from Arkansas; *Acer Saccharum*, *Quercus rubra*, *Hicoria ovata* and *Picea rubens* from Vermont; *Fraxinus Americana* and *Liriodendron Tulipifera* from New York; and *Larix Americana* from Michigan.

Further and more thorough tests were made later with a much larger number of woods, including most of the important timber species of the country. As will be perceived, those species which were utilized in the first experiment were also included in the later tests. The woods used in these last experiments are: *Sassafras Sassafras*, *Taxodium distichum*, *Nyssa aquatica* and *Liquidambar Styraciflua* from Arkansas; *Sequoia sempervirens*, *Sequoia Wellingtonia*, *Pinus Lambertiana*, *Pinus ponderosa*, *Pinus flexilis* and *Pseudotsuga mucronata* from California; *Catalpa speciosa* from Kansas; *Tilia Americana*, *Populus balsamifera*, *Larix Americana*,

* In naming the trees Professor Sargent's recently published Manual of the Trees of North America is followed.

Abies balsamea, *Pinus Strobus*, *Thuja occidentalis*, *Fraxinus nigra* and *Fagus Americana* from Michigan; *Pinus Taeda* and *Quercus rubra* from Mississippi; *Acer saccharinum*, *Cercis Canadensis*, *Pinus echinata*, *Cornus florida*, *Carpinus Caroliniana* and *Platanus occidentalis* from Missouri; *Juglans cinerea*, *Juglans nigra*, *Quercus Prinus*, *Ulmus Americana*, *Pinus divaricata* and *Hicoria ovata* from New Jersey; *Tsuga Canadensis*, *Picea rubens* and *Liriodendron Tulipifera* from New York; *Tsuga heterophylla* from Oregon; *Juniperus Virginiana* and *Nyssa aquatica* from Tennessee; *Pinus palustris*, *Ilex opaca*, *Magnolia glauca* and *Quercus Phellos* from Texas; *Acer Pennsylvanicum*, *Acer rubrum*, *Prunus serotina*, *Betula lutea*, *Acer Saccharum*, *Ostrya Virginiana* and *Salix* sp. from Vermont; and *Libocedrus decurrens* from Washington.

Attention should be given to the fact that much of the material used by Potter in his work was taken from branches and not from the main stems of the trees. Whether there is any difference between the branches and the stem with respect to the amount of unlignified cellulose present is as yet not definitely known; but the possbility of such a difference should be kept in mind.

CELLULOSE TESTS.

The examination for cellulose was at first performed simply with the microscopic sections after they were cut ready for use, but it was soon perceived that this method would not give accurate results. The samples of wood were then very carefully examined in all parts. If no cellulose was then found another search was made to make sure that it had not been overlooked at first. Thus the specimens which had no cellulose were really subjected to a much more rigid examination than were those which proved to have it.

Cellulose is said to occur in two ways in the fibers of wood in tree stems:* much more commonly as an inner, lining layer sometimes completely filling the cell lumen; and much more rarely as a layer situated between other lignified ones. Personal examinations have shown it to occur, with few exceptions, as an inner, supernumerary layer, but it has also been found that in exceptional cases nearly the whole secondary layer of the wall, which is usually completely lignified, gives a cellulose reaction although the reaction is more marked as we pass from the middle lamella to the cell lumen.

In the first lot of samples used for the preliminary tests it was found that cellulose occurred only in the wood of *Populus tremuloides* from New Mexico and in the wood of several trees of *Larix Americana* from the same locality in Michigan. In both cases the cellulose occurred only in some of the wood fibers and was present in the form of a lining, supernumerary layer of the wall.

In the more extended examinations made later cellulose was found in the wood of *Populus balsamifera* from the same locality as were the above mentioned trees of *Larix Americana* from Michigan. It may be added that a tree of *Populus tremuloides* from Vermont also showed large quantities of cellulose in the wood fibers. The occurrence of cellulose so markedly in all of the specimens of *Populus* wood available led to the suspicion that the species of the genus *Populus* might be characterized by this phenomenon wherever they might grow. Accordingly an effort has been made to obtain, as far as possible, specimens of all of the native species of this country. Of the eleven species which are listed by Sargent † in his latest publication the following have been examined: *Populus tremuloides*, *Populus balsamifera*, *Populus deltoidea*, *Populus trichocarpa* and *Populus acuminata*. Every specimen

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* Sanio. Bot. Zeitung. **1863**: 101–111. † Sargent. *l. c.*

examined has had cellulose very abundantly and in thick layers such as are shown to occur in the wood of *Populus tremuloides* in plate 1 figure 1, and of *Populus balsamifera* in plate 2, figure 2. Consequently there seem to be good indications that the genus *Populus* is characterized by the occurrence of cellulose in an extra layer.

A number of species of *Salix* from widely scattered localities have also been examined. *Salix alba* was found to have an inner lining layer of cellulose in some of the fibers of the wood. *Salix fragilis* had a very pronounced layer in stems about four years old. *Salix* sp. from California had cellulose in nearly all of the wood fibers. *Salix fluviatilis* from South Dakota had a very pronounced cellulose lining in many of the fibers. *Salix viminalis* has been found to have a thin layer of cellulose in the fibers.

Beside the above species of *Populus* and *Salix* which have been personally found to have unlignified cellulose there are a number of cases which other workers have noted. Sanio * found it to occur in wood of *Populus pyramidalis*. Sablon † found it in wood of *Salix* sp.

The sections of *Populus* and *Salix* were found to have the cellulose in the form of a thick and very distinct inner layer of the fiber wall which seemed to be somewhat loosely attached to the secondary lignified one. In some cases it filled the entire cell cavity, but this was not commonly true. The cellulose layer was quite sharply delimited from the lignified one. Cellulose was never present in the cells of the oldest wood of the annual rings, but was always situated in the more open early wood. It was found only in the wood fibers and only in those fibers which do not join or abut upon the vessels and tracheids.

In the *Larix* sections cellulose was present only in certain of the annual rings and also only in the wood fibers.

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* Sanio. *l. c.* † Sablon. Rev. gén. d. Bot. **16:** 360–363. (1904).

It consisted of an inner, lining, layer which was not as thick as was that of the *Populus* species.

Later and more protracted examination of the other woods showed cellulose to be present in small quantities in a single ring of the wood of *Acer Pennsylvanicum.* It was also noted in wood of *Acer saccharinum*, *Catalpa speciosa*, *Nyssa aquatica*, *Pinus Taeda*, *Platanus occidentalis* and *Ulmus Americana.* It was present in all of these only in small quantities.

BOILING TESTS.

The boiling tests were performed as follows: transverse and longitudinal sections of the wood were cut, placed in distilled water in sterile test-tubes, and boiled in an Arnold sterilizer for two or three hours per day in a manner similar to that commonly used in the discontinuous sterilization of culture media. Attention is here drawn to the fact that the temperature reached in the Arnold sterilizer is about 100 degrees Centigrade. The boiling was continued until a total of twenty hours had been attained. The tubes were then placed in an autoclave and steamed at a pressure of about sixteen pounds and a temperature of about 120 degrees Centigrade. This steaming in the autoclave was continued until forty hours of boiling had been accomplished.

After fifteen hours of boiling in the Arnold sterilizer a faint appearance of delignification was perceived in the sections of *Sassafras Sassafras.* Twenty hours of boiling made it very pronounced. See plate 2, figures 3 and 4. After seven hours in the autoclave, or a total of twenty-seven hours of boiling, the sections of *Picea rubens* also showed a light blue reaction in the inner lignified layer of the fiber walls when treated with chlor-iodide of zinc. See plate 2, figure 1. With these two exceptions the forty hours of boiling seemed to have no effect upon the

lignin of the sections. Potter does not state at what temperature his experiments were carried on but it is presumably at about 100 degrees Centigrade. If his results were applicable to woods in general the delignification in the present experiment should have been apparent even sooner than it actually was; and it should have taken place in all or nearly all of the woods instead of but two out of the twelve which were used. But we find none of the woods were delignified in the time stated by him to be effective with the woods tested by him. Apparently then the woods used in these experiments are more thoroughly lignified or else hold their lignin in combination more firmly than was the case with the woods used by Potter.

The later boiling tests were performed wholly with the autoclave at a temperature of about 120 degrees Centigrade. In general it may be said that many of the woods gave evidence of delignification after fifteen hours of treatment, and nearly all, after eighteen hours of steaming. *Sequoia Wellingtonia*, *Betula lutea* and *Juglans nigra* gave no results so far as could be detected, but it is barely possible that these woods are dark colored enough so that the reaction was not seen even if obtained.

SOLUBILITY OF LIGNIN IN SPRING AND SUMMER WOOD.

An experiment was next tried to test the relative solubility of the lignin of the early and late wood of the annual rings. For this purpose a wood was chosen which showed a decided difference between the two parts of the ring. *Pinus palustris* seemed to be very good, having a decided difference in the thickness of the fiber walls of the spring and summer wood, and also being entirely free of unlignified cellulose. Microscopic sections were cut from fresh wood and boiled in the manner above indicated. After a very long period of boiling, a record of which was unfortunately not kept, it was found that the spring wood seemed to be

wholly delignified, but the summer wood was still lignified, and so far as could be noted showed very little effect from the boiling. The various cellulose and lignin tests were used and invariably indicated the same results. See plate 1, figure 2. Thus it seems to be proved that the lignin of the spring wood is held in combination less firmly than is that of the summer wood. This is probably due to the greater thickness of the walls in the summer wood, as the difference in thickness between the two parts of the ring was very marked as will be readily perceived on referring to plate 1, figure 2. The results of this test seem to furnish a clue as to the reason why certain of the wood rotting fungi disintegrate the walls of the spring wood while the summer wood of the same ring is apparently unaffected.

Later tests with the various woods enumerated above have shown that what is true of the wood of *Pinus palustris* is also true of many others. The degree of differentiation between the two parts of the annual ring seems to be the controlling factor in the relative solubility of the lignin of those parts. In other words, those woods which have a decided difference between the spring and summer wood of the annual ring are almost sure to have the lignin extracted from the former quicker than it is from the latter: and those woods which have but slight differences between the two parts of the ring are apt to have the lignin extracted from all or nearly all of the ring at the same time.

ENVIRONMENT AND LIGNIFICATION.

Potter's results may be accepted as true for the woods used by him but they cannot be taken as significant with respect to woods grown under all conditions. The above experiments have shown that there is a decided difference between the results obtained by him and those of the present tests. As is well known, there is a very great difference in the environment of trees grown under different

conditions and in different climates. All workers with wood recognize the fact that its strength is a very variable quantity and for that reason it is unsatisfactory for use in heavy structures, since the amount of stress which it can withstand is more variable than is that of steel and iron. There is a decided difference between a certain species grown on dry hills and the same species grown in moist lowlands. Much more then must there be a difference between wood of the same species when grown in essentially different climates. May not, in the same way, the degree of lignification of the walls be affected by external conditions? Potter * says, "local conditions of soil and climate seem in some cases, to retard the complete development of the xylem, and thus render such trees constitutionally weak and very liable to attack." Von Schrenk † has also intimated something of a similar nature in connection with *Fraxinus Americana* when growing on the western border of its range. He says, "In the present instance it would seem, that there might be some relation between the greater susceptibility on the part of the ash near its western limit and its generally weaker development at this limit. It will be an interesting point to determine for instance whether the rate with which branch wounds or stubs heal in Ohio and Pennsylvania is greater than in Missouri and Kansas. That the rate of growth is slower in the Western States we know." In the same way may not the firmness with which the lignin is held in combination with the other constituents of the wall be also affected by the external conditions? This certainly does vary as is shown by the preceding experiment upon the spring and summer wood.

ARRESTED DEVELOPMENT AND RESERVE STORAGE.

The occurrence of cellulose in the form of an inner,

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* Potter. *l. c.*

† Von Schrenk. U. S. Dept. Agric. Bureau of Plant Industry. Bull. 32: 11. (1903).

supernumerary, lining layer of the fiber walls has been held by several botanists to be due to arrested development of the walls. It has seemed to the writer that there are certain facts in connection with cellulose thus situated which point directly against this theory. First, cellulose as above mentioned forms an *extra* layer of the wall. In other words the wall is completely formed and has, besides the usual layers, an extra one consisting of cellulose. Measurements of the fiber walls of *Populus balsamifera* show a difference of five microns in favor of the walls having the cellulose layer, over those which do not have that layer. Second, the wall with the cellulose layer is thicker than that without the cellulose, as is shown by the above measurements. It may also be stated that the difference in most of the woods was striking enough so that the observer could easily perceive without measuring that there was a decided difference between the two kinds of walls. Third, those fibers which are situated near vessels or tracheids do not usually have the cellulose layer. That is, those fibers which commonly do not have starch deposited within them are the ones which seem to be more apt to have the cellulose. This might be connected with the conversion of the starch into cellulose in such of the fibers as do not have the starch. Sablon* has proved that in willow wood the cellulose is plentiful when the starch has disappeared almost completely, and there thus seems to be a direct conversion of the starch into the cellulose. Fourth, the tendency is for the cellulose to be present in the spring wood and not in the summer wood of the annual ring where it would seem that arrested development would be most apt to affect the formation of the wall. There has been noted no indication of the final lignification of this cellulose layer, although Sanio † has stated that he noted such

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* Sablon. Revue générale de Botanique. **16**: 362. (1904).

† Sanio. *l. c.*

indications in certain stems which were especially favorable for the purpose. There certainly is still much doubt whether all the stems having this cellulose layer do finally become wholly lignified, and this seems especially true of the Salicaceae.

The exceptional cases mentioned above where the cellulose seemed to extend into the secondary layer (which is usually lignified), are not considered in this connection. Indeed they may very well be due to arrested development and are here so considered.

DELIGNIFICATION BY FUNGI.

Potter states that "the conclusion is inevitable that the occurrence of cellulose which has been attributed to the action of the fungi must to some extent be ascribed to conditions already present," implying that the cellulose which is found as a supernumerary lining layer of the wood fibers is the same cellulose which is found in woody tissues which are badly rotted by certain of the wood rotting fungi. He very evidently had in mind certain experiments of Ward * and Biffen † in testing the action of *Stereum hirsutum* and *Bulgaria polymorpha* upon wood when grown in pure cultures upon it. In both of these cases it happens that the delignification was apparently not very extensive and affected but a portion of the wall. In making the above statement Potter was at least partly correct in applying it to such cases, where the delignification was rather slight and affected but a portion of the wall. The statement should have been limited to such cases, as it is not applicable to those where the delignification has taken place throughout the whole wall as is the case in the last stages of disease caused by many of the wood rotting fungi, for the cellulose of normal wood occurs in such a

* Ward. Phil. Trans. Royal Soc. **189.** Series B. (1897).
† Biffen. Annals of Botany. **15**: 119–133. (1901.)

manner that it can have little or no effect upon the final result where the delignification is extensive, as is shown quite conclusively a little later in this paper.

The cellulose which is so often found in wood undergoing the last stages of decay is the cellulose skeleton or framework of the secondary, lignified layer which is universally present in old wood fiber walls, whether they have the supernumerary cellulose layer or not. That this is so is proved by a careful examination of the various stages of rot caused by any one of the numerous wood rotting fungi which delignify woody cell walls. The published work and drawings of Robert Hartig * alone are sufficient to prove this, as it has been found by the writer that cultures from rotted wood in the middle of a decayed stem of a living tree, if the exterior layers of wood are still alive, invariably yield but a single fungus, thus proving that the attacking fungus occurs in a pure state at least in most cases. If there is any cellulose layer present in the wood before it is attacked it is probably the first part of the cell wall to be dissolved and it is fairly certain that it does not exist in the last stages of decay.

Two instances of trees attacked by wood rotting fungi which fully bear out the last statement have recently come to the writer's attention.

A tree of *Carpinus Caroliniana* was found badly decayed by *Polyporus gilvus*,† which in this case seemed to be a wound parasite. At the edge of the affected wood was a narrow border of dark, infiltrated cells separating the healthy from the diseased wood. In the healthy wood just outside this infiltrated zone the wood fibers in many cases had an inner, lining layer of cellulose. This layer disappeared in the infiltrated zone while in the very badly

* Hartig. Wichtige Krankheiten der Waldbäume. 1–127. (1874); Die Zersetzungserscheinungen des Holzes der Nadelholzbäume und der Eiche. 1–151. (1878).

† Engler & Prantl's Pflanzenfamilien is followed in naming the fungi.

rotted tissues the cell walls consisted only of cellulose, the lignin appearing in greater and greater quantity as the healthy wood was approached. This single instance alone seems to prove that the cellulose layer of the healthy wood does not have any connection with the cellulose left by the action of the fungus upon the cell wall.

Still another instance was noted which seemed to confirm and make this point doubly certain. Sections of trunks of trees of *Populus tremuloïdes* rotted by *Fomes igniarius* were examined carefully for the occurrence of cellulose. It was found that the wood fibers of the healthy wood in most cases had an inner, supernumerary layer which turned blue with chlor-iodide of zinc. The rotted tissues were separated from the healthy ones by a narrow infiltrated zone. Just inside this zone of infiltrated cells the affected fibers were of a very uniform thickness and the walls were decidedly thinner than those of the healthy wood where the cellulose was present. In the rotted tissues the cell cavities were also larger than in the unaffected cells. In other words the cellulose layer is more or less completely dissolved from the interior of the fibers, and the secondary layer is delignified, while the cellulose skeleton is left until the last.

These two instances seem to show that there can be no doubt regarding the solution of the supernumerary cellulose layer during the early stages of decay caused by some of the fungi. It is believed that this will prove to be true of most if not all of them. Hartig very evidently understood this and for this reason gave no further attention to the occurrence of cellulose as an extra layer in the wood fibers of oak wood than to mention its occurrence.*

Actual measurements made in the wood fibers of the healthy and rotted wood of the same sections show beyond

* Hartig. Die Zersetzungserscheinungen des Holzes der Nadelholzbäume und der Eiche. 94. (1878).

any doubt that the thickness of the healthy wall is greater by a decided difference than is that of the cellulose fiber walls which are left in the badly rotted tissues. Such measurements have shown a difference of about a third of the total thickness of the original healthy wall between the two series. It has been stated distinctly by practically all workers with this group of fungi that delignification is a very common phenomenon as a result of the action of the mycelium of these fungi, which grows in the wood and disintegrates it to a greater or less extent as the case may be.

That cellulose is left by many of the fungi in the last stages of decay of the wood in which they are growing is but a very natural phenomenon to one who is familiar with the structure of woody cell walls. Chemical analyses have shown that cellulose is the basis of the structure of the wall and makes up a large percentage of it by dry weight. The methods of analysis are, to be sure, not satisfactory for exact quantitative results since each method gives results varying from the others by a small margin, yet the figures obtained may be used provided we keep in mind the fact that they are but approximate. Czapek * has given a summary of what has been done in this connection by different chemists. The following table shows the results obtained by two of the best methods of analysis.

	LANGE.	SCHULZE.
Beech	54.0-53.0-53.0	51.0-50.5-50.0
Fir.	51.0-50.0-50.6	48.0-48.2-49.0
Oak.	55.0-56.0-56.0	52.0-52.0-52.5

The figures indicate the percentage of cellulose found in the woods named by the two methods of analysis and also show the amount of variation in the results obtained. Analyses of pine wood showed that the percentage of cellulose varied from 47.5 to 53.5 per cent, and it was found that the sap wood was richer in cellulose than was the

* Czapek. Biochemie der Pflanzen. 1 : 563-564. (1905.)

heart wood. In general it may be said that from 45 to 60 per cent of wood is cellulose. Besides all this, pure cultures of several of the more common of the wood rotting fungi have been grown upon blocks of wood, which so far as could be determined, had no unlignified cellulose in the cell walls, and delignification has resulted while similar control blocks have shown no such delignification.

EFFECT OF STERILIZATION UPON WOOD BLOCKS.

When it is considered for how long a time the thin microscopic sections of the various woods were submitted to boiling at extreme temperatures before any decided effect could be detected one can hardly say that the effect of boiling at 100 degrees Centigrade for the time necessary for sterilization can be appreciable in cell walls in the interior of relatively large blocks of wood such as are commonly utilized for cultures of the wood rotting fungi. It seems that the effect upon the lignin of a block of wood which the investigator is sure has no unlignified cellulose originally, is so slight that it may be neglected. This is still more true when we remember that simple soaking has a more or less solvent effect upon the lignin in any method of culture work. There can be no doubt that when finely divided wood is soaked in water or is boiled, lignin is extracted in sufficient quantity for lignin reactions to be obtained in the filtrate from the extracted material. But this is very different from the detection of that extraction by micro-chemical examination of the treated walls themselves; for it must be confessed that micro-chemistry furnishes methods which are far from accurate for quantitative analysis.

In testing the action of fungi upon wood we must keep in mind the solvent action of boiling and of continual soaking in water and not be too hasty in drawing conclusions from meager results which might be confused with such action of boiling or soaking. Control tubes of wet wood

and careful examination of the material used should obviate much of this difficulty however.

ENZYMES OF FUNGI.

Potter also states that, "the delignification cannot be entirely attributed to an enzyme secreted by fungi." As above stated it has been shown that the original thickness of wood fiber walls is very materially reduced by some agent at the very time when the fungus is attacking the tissues in question. It is also shown that the cellulose which may or may not be present in the walls *before* they are attacked certainly is not the same unlignified cellulose which is present *after* the destruction of the wood by the fungus. It seems certain then that fungi do delignify woody cell walls.

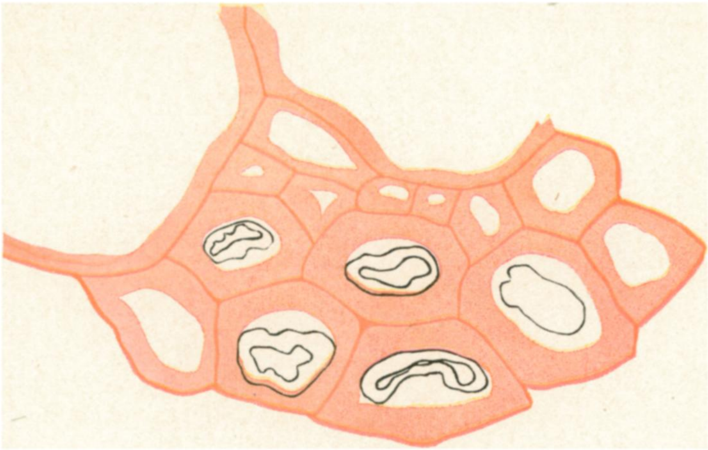
There can be no doubt that enzymes, or some substance exhibiting the characteristics of enzymes, have been proved to exist in some of the fungi and indeed in some of the wood rotting ones. Such investigations as have already been published in this connection would seem to prove beyond any reasonable doubt that enzymes are secreted by fungi and that they are of the most varied character. Diastase seems to be proved to be present in many of the different fungi and in the wood rotting ones. The disappearance of starch in the early stages of attack of some of the wood rotting fungi upon wood seems to be generally attributed to the secretion of diastase by the fungi. Why should the disappearance of lignin from the fiber walls, leaving cellulose in the last stages of decay, be attributed to any other cause than the secretion of a delignifying enzyme until we have proof of some other agent which is capable of such action? To be sure, the separation of an enzyme or a juice showing enzymic action from the fungus being studied is the exact proof of the existence of an enzyme; yet when we consider that enzymes are accepted

by practically all botanists as being present in fungi we can hardly come to but one conclusion regarding the existence of a delignifying enzyme.

EXPLANATION OF PLATES.

Plate 1. — 1, Portion of transverse section of wood of *Populus tremuloides* showing several wood fibers with a third, inner, cellulose layer. This layer is colorless, as the section is stained with phloroglucin which is a test for lignin. This supernumerary layer is characteristic of the species of the genus *Populus* so far as they have yet been examined, five of the endemic North American species from the various parts of the country having been tested. 2, Portion of a transverse section of wood of *Pinus palustris* showing one annual ring of growth. The thicker walled cells form the summer wood of the ring, while the thin walled ones make up the spring wood. This section has been boiled until the lignin was extracted from the spring wood, and was then stained with phloroglucin, a test for lignin. The red walls have reacted with the chemical showing that lignin is still present in the summer fibers. The yellow cells have retained their normal color to a greater or less extent and gave no reaction with the phloroglucin, thus showing that they have been delignified quite completely. The section shows the relative solubility of the lignin in the two parts of the annual ring.

Plate 2.—1, Portion of a transverse section of wood of *Picea rubens* boiled for 27 hours. This has been stained with chlor-iodide of zinc, a test for cellulose. The cellulose has been colored blue while the other parts of the walls are of their normal color. This shows the delignifying action of boiling water upon the lignin of the fibers. 2, Transverse section of wood of *Populus balsamifera* cut from a freshly felled tree and stained with chlor-iodide of zinc. The thick supernumerary layer of cellulose is colored blue. 3, Transverse section of wood of *Sassafras Sassafras* boiled 20 hours and stained with chlor-iodide of zinc. This is drawn with a smaller magnification than are the other figures. It shows the frequency of the cellulose layer, in the fibers of the wood. Note that there is no cellulose present in the last cells formed in the ring, which are in a vertical line near the left of the figure. 4, Transverse section of wood of *Sassafras Sassafras* drawn on the same scale as the other figures. This was boiled and treated with chlor-iodide of zinc and shows the character of the delignified layer in the fibers. Most of the fibers adjacent to the vessels and tracheids do not have the cellulose layer.



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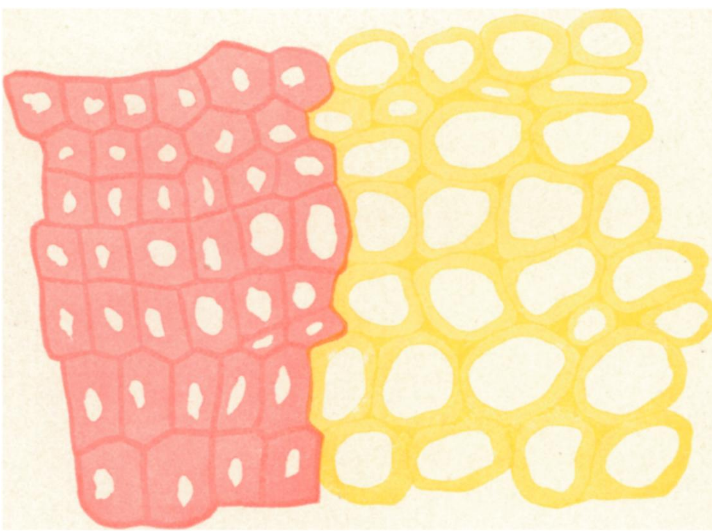

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LIGNIN AND CELLULOSE.

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